Studies on Luminescence in Marine Snails

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Among the Gastropoda Opisthobranchiata certain genera of the Polyceridae, Tethyidae, and Phillirhoidae are luminous. Among the Gastropoda Pulmonata two luminous species are known: *Latia neritoides*, fresh water limpets, first discovered in New Zealand by Suter (1890), and *Dyakia striata*, land snails, found by myself in Singapore in 1946.

Luminous species of Gastropoda Prosobranchiata are very rare, however, according to E. N. Harvey in his book *Bioluminescence* (1952). Turner in 1948 informed him by letter that *Tonna galea* Linné, a marine snail, is luminescent. When this snail moves through water with its foot well extended, it emits a greenish-white light. A species of doubtful luminescence is also found among the heteropods (Pterotracheata) according to Keferstein in Bronn's *Tierreich* (1862–1866).

We have found two species of luminous marine snails on Borawazawa Beach, at Suyeyoshi Village, which is located on Hachijo Island, 157 miles south of Tokyo. This discovery was made during ebb tide on April 23, 1953. While I was strolling on the beach with an acquaintance, Mr. H. Okuyama, teacher of Suyeyoshi Primary School, he chanced to raise a stone, and we were both astonished to find under it some small marine snails which were emitting light as they rolled in the water. Excitedly we collected many specimens, examining them closely to find out if these marine snails were really luminous, or whether their luminescence was due only to their having eaten some luminous matter or to infection with luminous bacteria.

As a result of subsequent experiments, I decided that these snails were true luminous animals, possessing luminous organs on their mantles (Haneda, 1955).

During a trip to the Luminescence Conference at Pacific Grove, California, for the meeting of March 29–April 1, 1954, I had the opportunity to continue my studies of these interesting animals. I collected another species of luminous snails on Waikiki beach, Oahu, and one other species on Onekahakaha beach, Hawaii, and on the beach at Key West, Florida (see Haneda, 1955).

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I would also like to express my warmest appreciation to the Committee of the Luminescence Conference, for inviting me to participate in the meetings of March 29–April 1, 1954 at Pacific Grove, California, which gave me the opportunity to collect many American specimens.

MATERIALS

The luminous marine snails which I have collected all belong to the genus *Planaxis* of the Planaxidae and are comparatively small. The various species of this genus in which I have observed luminescence are shown in Table 1.

All of these animals are capable of living in aquaria for a long time, usually up to two to three months, and in some cases as long as one year. I collected many specimens in April at Hachijo Island and brought them to my laboratory in a small bottle. These animals lived in a small dish until the end of August of the same year, during which period the water was changed several times. I twice air mailed several living specimens of *Planaxis virgatus* in a small bottle of sea water to Dr. E. N. Harvey of Princeton University. The first time all the specimens were dead when Dr. Harvey received them. But the second time the specimens arrived alive.

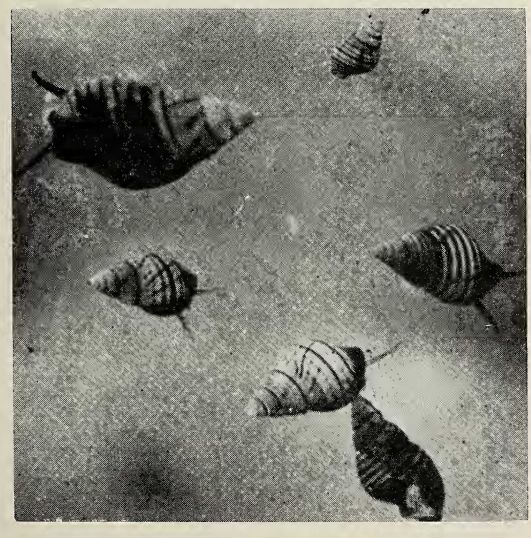

FIG. 1. *Planaxis virgatus.*

FIG. 2. *P. labiosus.*

I brought some specimens of the Hawaiian species in a small bottle of sea water to the Conference on Luminescence and demonstrated their luminosity to Dr. Harvey and other members present. Afterward I took those specimens on my journey in the United States and brought them back to my laboratory about two months later. The snails lived in a small aquarium until March 21, 1955, and some of them hatched larvae, but luminosity in these larvae was not observed.

OBSERVATION OF LUMINOUS PHENOMENA

Under natural conditions, the light of these animals cannot be seen well; they will emit light only under strong stimulation. If many specimens are placed in a bottle and are well shaken in the dark, some of them become luminous and twinkling. The light usually continues one or two minutes after stimulation, then gradually disappears. But if the body of the snails is irritated, the light re-

TABLE 1
DATE AND PLACE OF COLLECTION OF THE VARIOUS SPECIES OF THE GENUS *Planaxis* AND THEIR SIZE AND REMARKS

SPECIES	PLACE	DATE	SIZE OF SHELL		REMARKS
			Height, mm.	Diameter, mm.	
Planaxis virgatus Smith...	Borawazawa Beach, Hachijo I., Japan	April, 1953	8.0	4.0	Luminous
	Habu, Ohshima I., Japan	Oct. 1953			
P. periscelida Dall.....	Heta, Izu Peninsula, Japan	May, 1953	3.5	2.0	Luminous
P. longispira Smith.....	Dogashima, Izu Peninsula Japan	Aug., 1953	6.0	2.8	Luminous
P. lineatus da Costa.....	Waikiki Beach, Honolulu, Hawaii	March, 1954	10.0	5.2	Luminous
P. labiosus A. Adams....	Onekahakaha Beach, Hilo, Hawaii	March, 1954	10.0	5.0	Luminous
P. labiosus A. Adams....	Key West, Florida	April, 1954			
P. sulcatus Born.....	Shirahama, Wakayama Pref., Japan	April, 1955	15.0	10.0	Non-luminous

appears once again. After crushing the shell and removing the body of the snail, a low power magnification will show that the luminous part of its body is situated on the mantle in a limited area (Fig. 3). When the body is put into fresh water, the light on the mantle continues as a more prolonged glow. However, after 10 or 15 minutes in fresh water, the animal becomes weak and its light cannot be seen even after strong stimulation. If the body is placed on photographic film for a few minutes in the dark, an image of the light appears.

Observed under the microscope in the dark, the luminous area of the snail takes on the appearance of a night sky. Minute luminous points glimmer and twinkle like so many stars. The luminescence of this animal is intracellular; no luminous slime goes into the surrounding sea water. Among the six species of Planaxidae, which were examined, the following five have the same type of luminous organ: *Planaxis virgatus*, *P. longispira*, *P. periscelida*, *P. lineatus*, and *P. labiosus*. *P. sulcatus*, however, has no luminous organ at all.

EFFECTS OF TEMPERATURE

No effects are noticable to the naked eye in the intensity of luminescence in marine snails under thermostimulation. Through the use of the highly sensitive 1921 photomultiplier, followed by two stages of amplification feeding into a Brown recorder, however, the light intensity changes resulting from changes in temperature are readily discernible. I had the opportunity to make use of such equipment through the kind offices of Dr. Bernard L. Strehler of the Department of Radiobiology at the University of Chicago when I visited that institution in April, 1954. Our tests were extremely interesting. Contained in a small glass tube of sea water at a temperature of 15° C., the snails were placed near the photomultiplier and the temperature of the tube was raised and lowered in two successive cycles and the light intensity recorded.

From an initial centigrade reading of 15° C. the snails' temperature was raised to 45° C. and lowered to −10° C. in a period of less than 10 minutes. From this point the temperature was again raised gradually to 60° C. and lowered to 15° C. During the first cycle the maximum of relative light intensity was at 45° C. As the temperature decreased so did the intensity, until at −10° C. it could not be detected.

During the second cycle, however, the in-

tensity became progressively weaker after 30° C., despite an increase of temperature to 60° C. At this point the intensity had dropped to zero, and during the following decrease in temperature, it showed no discernible recovery. As far as could be determined, the snails had died at approximately 60° C.

STRUCTURE OF THE LUMINOUS ORGAN IN *Planaxis labiosus*

The luminous organ, situated on the dorsal part of the mantle in the limited area shown in Figure 3, is a translucent pale blue color when fresh. The external visible area of this luminous organ consists of many folds of luminous tissues which run parallel with each other. If the body is taken out of the shell and put into an aluminum morine solution (diluted 500,000 times) for a few minutes and then washed carefully in fresh water, it is possible, by observation with Yasaki's fluorescent microscope (1952), to see the beautiful blue-green fluorescent light which these luminous tissues emit. The structure of all five luminous species of *Planaxis* is practically the same: the luminous cells are restricted to this area of the mantle.

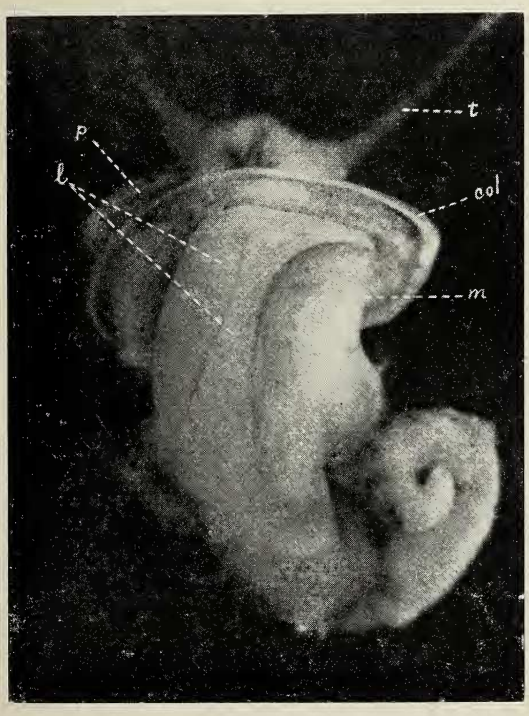

FIG. 3. Dorsal view of the body of *Planaxis labiosus*, removed from the shell. *l*, Luminous area of mantle; *m*, mantle; *col*, collar; *p*, propodium; *t*, tentacles.

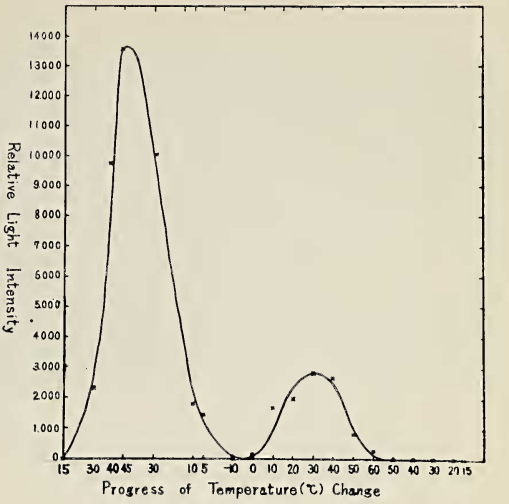

FIG. 4. Effect of temperature on *Planaxis labiosus* luminescence. (Numbers on the vertical axis are counts of the recorder of the photomultiplier.)

HISTOLOGY

Since the luminescence of this snail is localized in a limited area on the mantle and the animal is small in size, the whole body was preserved in a fixative of sea water prepared with formalin and Bouin's solution. The material was cut in 10 μ sections both longitudinally and transversely. The stains used were haematoxylineosin and aluminum morine. The appearance of the longitudinal section of the mantle is much like that of a comb. On the inside of the mantle, where the luminous area is situated, there appeared many pleats arranged in parallel, but it was impossible to determine whether or not these pleats contain luminous cells. However in the luminous area of the mantle, under the thin epithelium, there appeared many cellular masses, which very probably contain the photogenic tissues of this body. Each of these masses consists of small, closely packed cells.

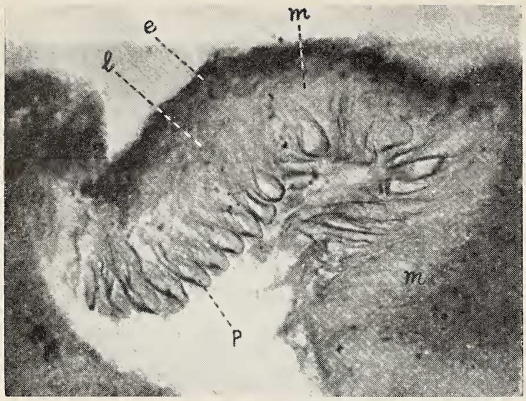

FIG. 5. Longitudinal section of the mantle containing luminous tissue of *Planaxis labiosus*. *l*, Luminous tissue; *m*, mantle; *p*, pleats; *e*, epithelium.

The character of these cells indicates that the luminescence must be intracellular.

BIOCHEMISTRY

Further experiments to relate luminescence to biochemistry were carried out as follows. A number of the snails were removed from their shells and thoroughly dried. They were then ground in a mortar and moistened with water, but luminescence did not appear. A negative luciferin-luciferase reaction was obtained by mixing hot water and cold water extracts of the crushed bodies. If the cold water extract of crushed luminous snails is allowed to stand until the luminescence of the extract disappears, it will not recover its luminescence even when ATP (Adenosinetriphosphate) is added.

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